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Acute and subchronic toxicity effect of *Momordica charentia* methanol leaf extract in albino rats

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ABSTRACT

Indigenous plants with medicinal properties have long been used in different folkloric systems globally. However, there is significant requirement to provide scientific explanation on the toxicological effect of medicinal plants before accepting them in folkloric system. This article is aimed to evaluate acute and subchronic toxicity of methanol leaf extracts of *M. charantia*. Phytochemicals screenings were assayed using standard methods, while acute and subchronic oral toxicity of was evaluated in albino rats as per OECD guide line. Phytochemical analysis of extract showed the presence of, alkaloids, flavanoids, phlobatannin, saponins, glycosides, terpenoid and Phenols, the LD50 of extract is above than 5000 mg/kg. Subchronic oral toxicity assayed in liver revealed a significant reduction (P<0.05) in AST in all the treated groups when compared to normal control group except group treated with 1500mg/kg which it's reduction is not significant (P>0.05), ALT showed a significant increase (P<0.05) in all the treated groups except group treated with 500mg/kg which it's reduction is not significant (P>0.05) compared to the normal control group. ALP biomarkers on the other hand significantly (P<0.05) increases in groups treated with 250and 500mg/kg compared to normal control group while group treated with 1000mg/kg significantly decrease (P<0.05) when compared with control group. ALB and TP revealed significant (P<0.05) decreases across all groups compared to control. However, total bilirubin and direct bilirubin revealed significant (P<0.05) increases only at groups treated with 250mg/kg respectively. While in results for kidney function, Urea revealed significant increase (P<0.05) in groups treated with 500, 100 and 1500mg/kg compared to control. Creatinine and uric acid significantly increase (p>0.05) only at group treated with 250mg/kg and 1000mg/kg compared to control respectively. Na+ significantly (P<0.05) increases across all extract treated groups compared to control. However there was no significant (P>0.05) deference in K+ of all extract treated compared to control. While, Cl- revealed significant decreases at groups treated with 500 and 1500mg/kg compared to control. However Urea and Uric acid showed significant increases at groups treated the higher doses when compared to control. In haematological indices (WBC), (LYM), (GRA), (RBC), (HGB), (MCV), MCHC, PLT and PCT parameters does not significantly (P<0.05) differ compared to control. In conclusion, with regard to the findings of the present studies the subchronic oral

administration of *M. charentia* extract for 28days does not cause any toxic effect on liver enzymes, kidney function parameters, as well as heamatological parameter.

Keywords: Toxicity, Acute, Subchronic, *Momordica charentia*, liver, kidney.

1. INTRODUCTION

In African, plants with medicinal properties have long been used in different folkloric systems globally (Jamshidi-Kia *et al*., 2018; Raju & Raju, 2020). The historic use of medicinal plants for treating numerous diseases evolved out of necessity and experience (Meuss, 2000). Many plants are rich source of a wide variety of secondary metabolites and interestingly many of the synthetic drugs that are currently are derived from herbs (Efferth and Koch, 2011). Regardless of how they are utilized, the fundamental problems faced by traditional medicinal is the lack of scientific synchronization for their mechanism of action and safety profile *in vivo (*Bernstein *et al*., 2021).

Therefore, it is vital to conduct potential toxicity studies based on accepted scientific guidelines to support the safety of medicinal plants used in various traditional practices. In addition, all herbal drugs prepared singly and or mixture of different medicinal plants must be scientifically validated for their safety consumption. However, there is significant requirement to provide scientific explanation on the toxicological effect of medicinal plants before accepting them in folkloric system.

Momordica charantia, commonly known as bitter melon, is a plant from the family *Cucurbitaceae* known over 100 years for its medicinal, pharmacological, and nutritional properties (Bortolotti *et al*., 2019). Due to detection of several phytochemical and bioactive compounds, majority of which has potent medicinal properties, this plant is used in herbal medicine globally for the treatment of numerous pathogens, diabetes, cancer, and other inflammation-associated diseases (Bortolotti *et al*., 2019). However, adequate toxicity studies on bitter melon have not been thoroughly documented. This paper is aimed to provide an insight on toxicological effect of *Momordica charentia* methanol leave extract on animal model.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Sample

The plant sample was collected in July 2021 from Birnin Kebbi town, Birnin Kebbi Local Government Area, Kebbi State, Nigeria. It was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro, with a voucher specimen (KSUSTA/PSB/H/VOUCHER/SN) deposited in the herbarium of the same Department.

2.2 Plant Preparation and Extraction

The leaves of *Momordica charentia* were washed with clean water and allowed to dry under shade for two weeks. It was then grinded to coarse powder using mortar and pestle. One thousand five hundred grams (800g) of the powdered leaves were soaked in 25000mls of methanol for 72hrs (Dupont *et al*., 2002). Then filtered using muslin cloth, the filtrate afterward was evaporated using oven at a temperature of 45^{o}C and after drying, the extract was stored tightly in a container and refrigerated at 4^{o}C. The percentage yield of the extract was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

2.3 Experimental Animals

The albino (Wistar) rats used in this study were purchased from Animal House, Usmanu Danfodiyo University, Sokoto in November, 2021. The animals were transported in rubber cages to Animal House, Faculty of Science, Kebbi State University of Science and Technology, Aleiro. The animals were allowed to acclimatize for two (2) weeks and were fed with standard rodent pellets starter (Chikum brand) also the animals were allowed access to water *ad libitum.*

2.4 Phytochemical Screening

Phytochemical screening was carried out according to the methods described by Harbone, (1973) and Trease and Evans, (1989).

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2.5 Acute Oral Toxicity Studies (LD50)

The acute oral toxicity study was conducted in accordance to Organization for Economic and Cultural Development for testing of chemical (OECD, 2001). The method has two phases, phases 1 and 2 respectively.

Phase 1

Nine (9) animals are required in this phase and are divided into three groups of three animals each. And each group were administered (10, 100 and 1000 mg/kg respectively), of test substance. Then the animals were further observed for signs of toxicity and mortality within 24 hours.

Phase 2

In this phase, three animals are used, and are divided into three groups of one animal each. They are administered with higher doses (1600, 2900 and 5000 mg/kg) of the extract and further observed for 24hrs for behavior and mortality). The animals were observed for signs of drowsiness, hair loos, and loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity (Lorke, 1983).

2.6 Sub-chronic Oral Toxicity Study

Fifteen (15) albino rats weighing100-250g were randomly distributed into five (5) groups of four (4) animals each. The animals were acclimatized for 2 weeks on a regular feeds with pelletized growers fed and water. Group one [control] was administered with distilled water alone, other groups (2-5) were administered 250, 500, 100 and 1500g/kg body weight respectively. The animals were administered at different doses of the extract for 28 days based on varying individual body weight as follows:

Group I	5ml/kg body weight of distilled water alone.
Group II	250mg/kg body weight of plant extract + distilled water
Group III	500mg/kg body weight of plant extract + distilled water
Group IV	1000mg/kg body weight of plant extract + distilled water
Group V	1500g/kg body weight of plant extract + distilled water

The extract was administered orally to the animals daily for 28 days. The rats were sacrificed on the 29th day of the experiment. Blood samples were collected in heparinized containers for biochemical analysis.

2.6.1 Measurement of Liver Function Test

Alkaline phosphatase activity was calculated according to the method described by Sood, (1999). Aspartate aminotransferase and alanine aminotransferase activity were calculated according to the method described by Reitman and Frankel, (1957). Albumin was calculated using bromocresol green method as described by Doumas et al., (1971). Total protein was estimated using the Biuret reaction method described by Lowry et al., (1951). Total and Direct Bilirubin were determined adopting the calorimetric method described by Jendrassik and Grof, (1938).

2.6.2 Measurement of kidney Function Markers

Serum urea was determined using the Berthelot colorimetric according to the method described by Young, (1937). Serum creatinine was determined using Jaffe's method as described by Bartels and Bohmer, (1971). Serum uric acid concentration was determined using the method described by Henry et al., (1957). Serum sodium and potassium ions were measured using flame photometry (Cheesbrough, 1991). Serum bicaronate and chloride ions were measured using titration/volumetric method (Chapman, 1961).

2.6.3 Haematological Analysis

Hematological parameters via, white blood cells count (WBC), red blood count (RBC) lymphocytes, hemoglobin, granulocytes, packed cell volume (PCV), neutrophils, (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets and Procalcitonin were analysed using an automated hematological analyzer Sysmex XS800i (Sysmex corporation, USA) (Theml et al., 2004).

2.7 Histopathological Examination

Histopathology was done using the method of Drury et al., (1967). Liver and pancreas of the rats were harvested and preserved in 10 % formalin. The organs were fixed in 10% formalin for 72 hours. The tissues were then dehydrated in alcohol of graded

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concentrations and embedded in paraffin. Tissues were sliced into sections of 5 µm thick and these were stained with hematoxylin and eosin for photo microscopic assessment. The slide was mounted on an electric light microscope for examination of any possible histopathological features. Photomicrographs of the samples were then taken.

2.8 Data Analysis

All data are presented as Mean ± SEM and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using (SPSS) version 20. Values are considered statistically significant at $P<0.05$.

3. RESULTS

3.1 Phytochemical Analysis

Phytochemical analysis of methanolic extract of *Momordica charentia* leaves revealed the presence of alkaloids, flavanoids, phlobatannins, saponins, glycosides, Phenols and terpenoid (Table 1).

Table 1: Results for Phytochemical Analysis

PHYTOCHEMICALS	RESULTS
Alkaloids	+
Flavonoids	+
Tannins	-
Phlobatanins	+
Saponin	+
Glycoside	+
Phenols	+
Terpenoids	+
Anthraquinone	-

KEY: + = Present, - = Not detected

3.2 Acute Toxicity (LD50) Profile

There was no any visible or detectable sign of toxicity even at the maximum dose limit (5000 mg/kg). Since no any apparent sign of toxicity or mortality recorded, the LD50 is assumed to be greater than 5000mg/kg b.w.

3.3 Sub-chronic Toxicity Profile of *Momordica charentia* Methanol Leaf Extract

3.3.1 Effect of M. charentia Methanol Leaf Extract on Bodyweight

The bodyweight of albino rats treated with *M. charentia* methanol leaf extract for 28 days showed a weekly increase in body weight in all groups from initial week (0) to last week of the experiment (week 4) Figure 1.

3.2. Effect of *M. charentia* Methanol Leaf Extract on Liver Function Parameters

The liver biomarkers of toxicity assayed revealed a significant reduction ($P<0.05$) in AST in all extract treated groups when compared to the normal control group except for the group treated with 1500mg/kg which it's reduction is not significant ($P>0.05$), ALT showed a significant increase ($P<0.05$) in all the treated groups except for the group treated with 500mg/kg which it's reduction is not significant ($P>0.05$) compared to the normal control group. ALP biomarkers on the other hand significantly ($P<0.05$) increases in groups treated with 250and 500mg/kg compared to normal control group while group treated with 1000mg/kg significantly decrease ($P<0.05$) when compared with control group (Table 2). ALB and TP revealed significant ($P<0.05$) decreases across all groups compared to control. However, total bilirubin and direct bilirubin revealed significant ($P<0.05$) increases only at groups treated with 250mg/kg respectively.

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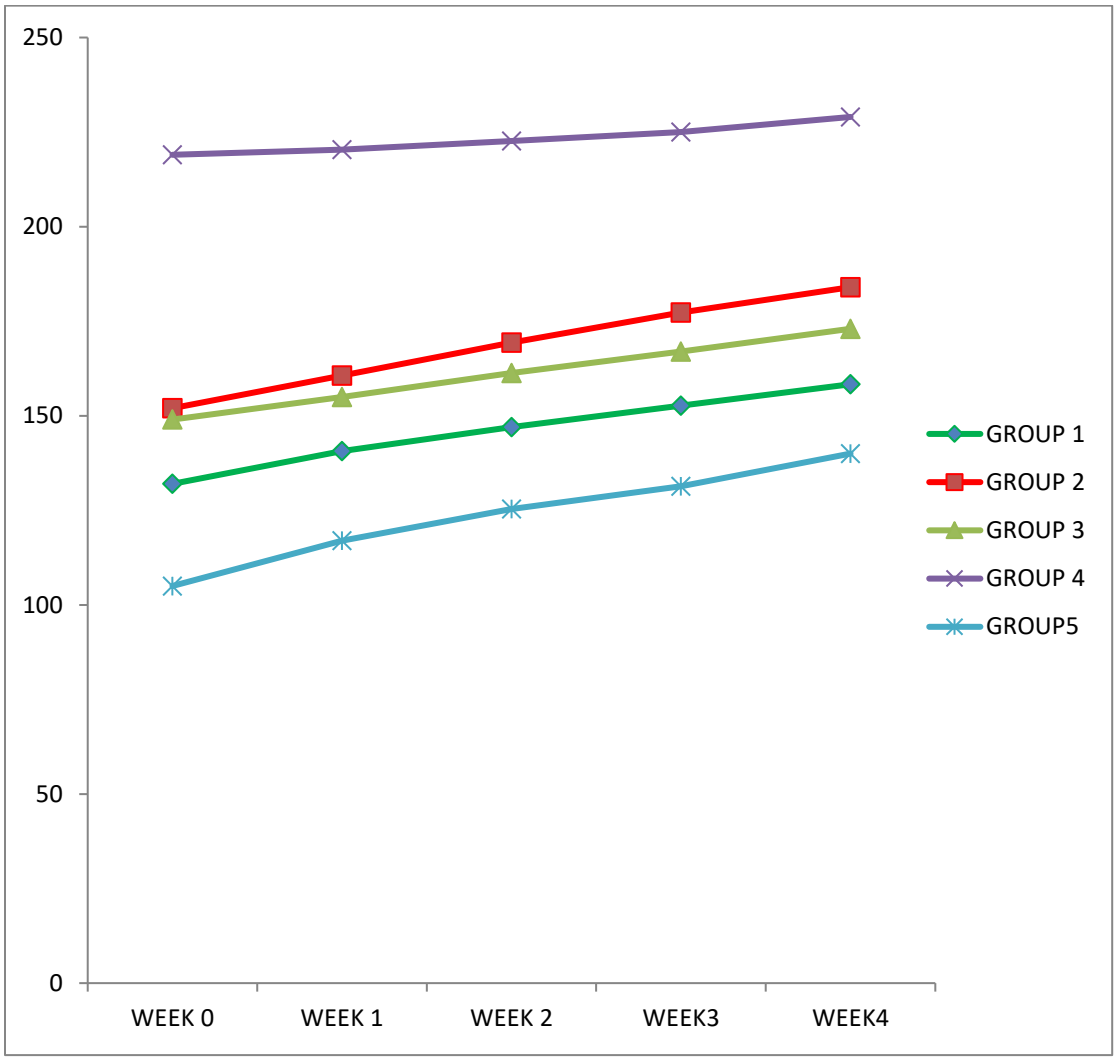

Figure 1: Weight of Animals Administered with *M. charentia* Methanol Leaf Extract four weeks.
{Group 1(normal control), group 2 (250mg/kg b.w), group 3 (500mg/kg b.w), group 4 (1000mg/kg b.w) and group 5 (1500mg/kg b.w)}.

3.2.4 Effect of *M. charentia* Methanol Leaf Extract on Kidney Function Parameters

The results for effect of *M. charentia* methanol leaf extract on liver function parameters are present in Table 3. Urea revealed significant increase (P<0.05) in groups treated with 500, 100 and 1500mg/kg compared to control. While, creatinine and uric acid significantly increase (P>0.05) only at group treated with 250mg/kg and 1000mg/kg compared to control respectively. Na+ significantly (P<0.05) increases in all groups treated with extract compared to control. However there wasn't significant (P>0.05) deference in K+ of all extract treatment compared to control. While, Cl- revealed significant decreases at groups treated with 500 and 1500mg/kg compared to control.

3.2.5 Effect of *M. charentia* Methanol Leaf Extract on Haematological Indices

The results for effect of *M. charentia* methanol leaf extract on haematological indices are present in Table 4. White blood count (WBC), lymphocytes (LYM), granulocytes (GRA), red blood count (RBC), haemoglobin (HGB), mean cell volume (MCV), MCHC, PLT and PCT parameters does not significantly (P>0.05) differ compared to control.

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Table 2: Effect of *M. charentia* Methanol Leaf Extract on Liver Function Parameters

Parameter	Control	(250mg/kg)	(500mg/kg)	(1000 mg/kg)	(1500 mg/kg)
AST (U/L)	491.30±3.89^{e}	246.20±5.65^{b}	393.83±1.27^{d}	196.10±7.77^{a}	344.43±6.58^{c}
ALT (U/L)	104.17±12.49^{a}	156.20±1.73^{c}	105.93±2.94^{a}	125.07±22.19^{b}	157.53±1.34^{c}
ALP (U/L)	89.20±7.84^{b}	166.47±4.00^{c}	146.23±8.88^{c}	65.30±6.64^{a}	68.97±4.76^{ab}
ALB (G/l)	4.27±0.03^{e}	1.87 ±0.33^{b}	1.73±0.33^{a}	2.17±0.33^{c}	2.30±0.00^{d}
TP (G/l)	10.53±0.67^{e}	5.60±0.06^{a}	5.90±0.00^{b}	6.83±0.03^{d}	6.07±0.07^{c}
TB (mg/dL)	7.97±0.12^{b}	14.13±0.07^{c}	7.77±0.07^{b}	2.20±0.07^{a}	7.87±0.07^{b}
DB (mg/dL)	1.87±0.03^{b}	2.50±0.06^{c}	2.10±0.06^{bc}	1.00±0.00^{a}	1.64±0.37^{b}

Values are presented as mean ± SEM (n = 3) value having similar superscript in rows are not significantly different at (P<0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin and DB- Direct Bilirubin

Table 3: Effect of *M. charentia* Methanol Leaf Extract on Kidney Function Parameters

Parameter	Control	(250mg/kg)	(500mg/kg)	(1000 mg/kg)	(1500 mg/kg)
Urea (mmol/l)	54.33±0.32^{b}	52.67±3.60^{b}	41.17±2.41^{a}	41.50 ±1.93^{a}	35.70±0.62^{a}
Creatinine (mg/dl)	84.63±1.09^{a}	168.50±1.57^{b}	86.67±37.66^{a}	67.73±9.77^{a}	80.70±1.46^{a}
Uric acid (mg/dl)	2.47±0.09^{d}	2.23±0.09^{c}	1.80±0.06^{b}	3.20±0.06^{e}	1.40±0.06^{a}
Na^{+} (mmol/l)	91.67±1.20^{a}	161.00±2.08^{c}	120.00±1.73^{b}	158.00±3.61^{c}	181.00 ±2.08^{d}
K^{+} (mmol/l)	100.00±2.89^{ab}	95.67±2.03^{a}	105.00±2.87^{b}	98.00±2.65^{ab}	98.00±1.15^{ab}
Cl^{-} (mmol/l)	19.00±0.58^{cd}	19.67±1.20^{d}	15.00±0.58^{ab}	16.67±0.88^{bc}	12.33±0.88^{a}

Values are presented as mean ± SEM (n = 3) value having similar superscript in rows are not significantly different at (P<0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. Potassium (K^{+}), Sodium (Na^{+}), Bicarbonate (HCO_{3})

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Table 4: Effect of *M. charentia* Methanol Leaf Extract on Haematological Indices

Doses (mg/kg)	WBC (x10⁹/L)	LYM (g/dL)	GRAN (%)	RBC (%)	HGB (%)	MCV (%)	MCHC (%)	PLT (%)	PCT (%)
Control	2.00±0.13[a]	354.30±267.89[a]	1.63±1.48[a]	7.64±0.12[a]	15.50±0.50[a]	74.47±1.58[a]	27.33±0.67[a]	680.00±83..37[a]	0.60±0.06[a]
250	4.28±0.90[a]	89.60±1.79[a]	0.26±0.16[a]	8.10±0.73[a]	15.33±0.59[a]	78.30±10.15[a]	27.97±0.74[a]	595.67±146.17[a]	0.49±0.10[a]
500	5.39±2.06[a]	90.70±2.28[a]	0.28±0.18[a]	7.02±0.41[a]	14.13±0.76[a]	74.70±1.90[a]	26.97±0. 58[a]	671.67±15.30[a]	0.55±0.03[a]
1000	4.23±0.61[a]	87.10±6.13[a]	0.32±0.19[a]	7.05±0.94[a]	14.13±1.73[a]	73.13±0.84[a]	27.47±0.60[a]	683.67±200.88[a]	0.55±0.14[a]
2000	3.40±0.47[a]	87.77±0.33[a]	0.16 ±0.02[a]	7.78±0.27[a]	15.23±0.47[a]	72.77±2.34[a]	27.00±0. 47[a]	657.33±34.60[a]	0.54±0.03[a]

Values are presented as mean ± SD (n = 3) value having same superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. WBC- White Blood Count, LYM- Lymphocytes, GRAN= Granulocytes, RBC= Red Blood Count, HGB- Haemoglobin, MCV= Mean Cell Volume, MCHC= Mean Corpuscular Heamoglobin Concentration, PLT= Platelet, PCT= Procalcitonin

3.2.6 Toxicological Effect of *M. charentia* Methanol Leaf Extract Liver and Kidney Tissues

Histopathological results showed that the liver of rats in normal control group and groups treated at 250, 500, 1000 and 1500mg/kg of *M. charentia* methanol leaf extract all exhibited normally distributed portal triad, central vein and hepatocytes (Plate 1-5). While kidney section of control group and groups treated at 250, 500, 1000 and 1500mg/kg of *M. charentia* methanol leaf extract all showed normal capsular space and regular glomerulus (Plate 6-10).

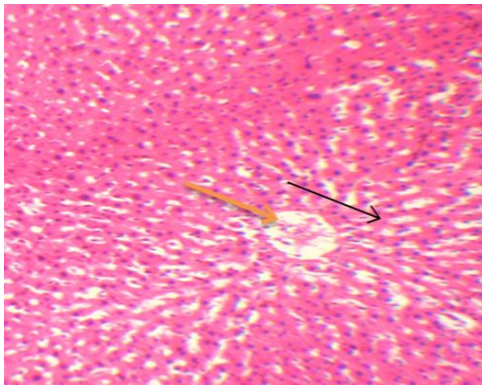

Plate 1: Photomicrograph of rat's liver obtained from control

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

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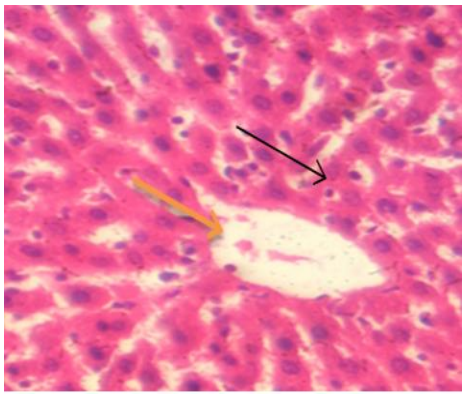

Plate 2: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg *M. charentia* Methanol Leaf Extract (H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

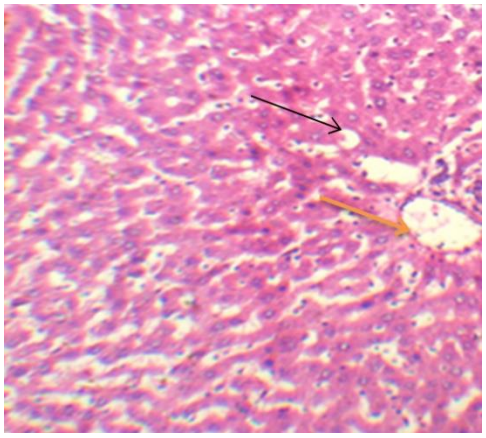

Plate 4.3: Photomicrograph of rat's liver obtained from group administered with 500 mg/kg *M. charentia* Methanol Leaf Extract (H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

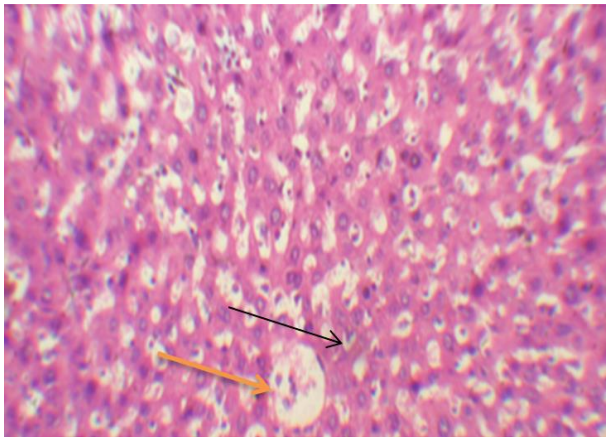

Plate 4: Photomicrograph of rat's liver obtained from group administered with 1000 mg/kg *M. charentia* Methanol Leaf Extract (H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

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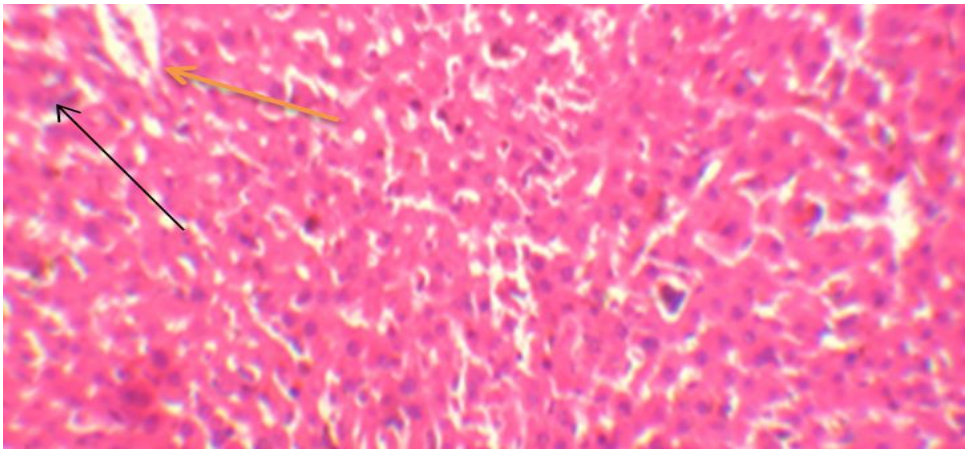

Plate 5: Photomicrograph of rat's liver obtained from group administered with 1500 mg/kg *M. charentia* Methanol Leaf Extract
(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

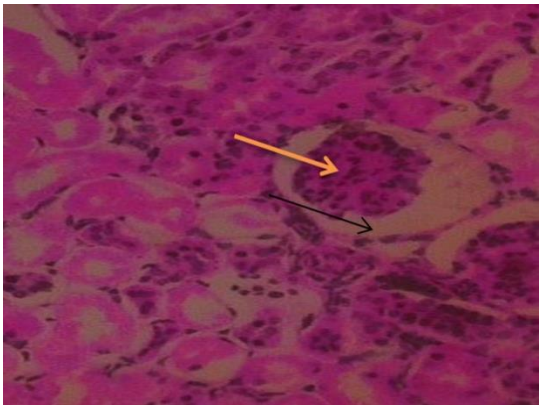

Plate 6: Photomicrograph of rat's kidney obtained from control
(H and E stain, x 100 magnification). Showing normal capsular space (black arrow), regular glomerulus (red arrow)

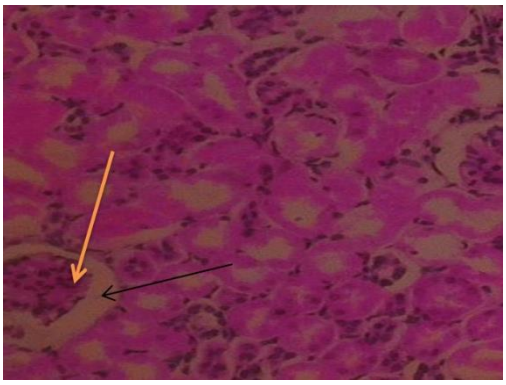

Plate 7: Photomicrograph of rat's kidney obtained from group administered with 250 mg/kg *M. charentia* Methanol Leaf Extract
(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

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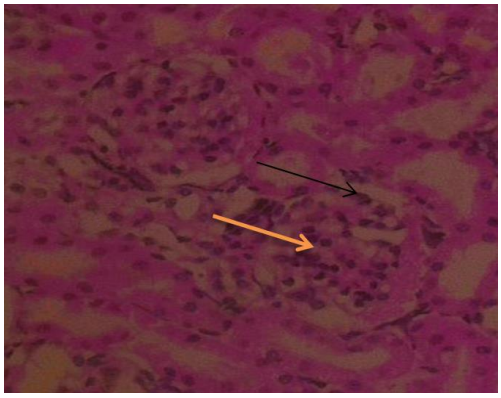

Plate 8: Photomicrograph of rat's kidney obtained from group administered with 500 mg/kg *M. char entia* Methanol Leaf Extract
(H and E stain, x 100 magnification). Showing normal capsular space (black arrow), regular glomerulus (red arrow

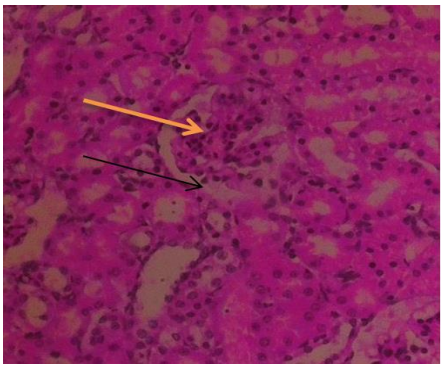

Plate 9: Photomicrograph of rat's kidney obtained from group administered with 1000 mg/kg *M. charentia* Methanol Leaf Extract
(H and E stain, x 100 magnification). Showing normal capsular space (black arrow), regular glomerulus (red arrow

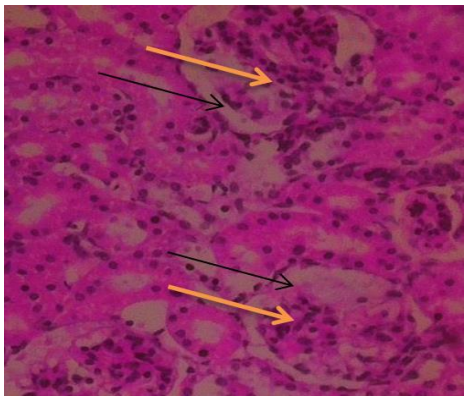

Plate 4.10: Photomicrograph of rat's kidney obtained from group administered with 1500 mg/kg *M. charentia* Methanol Leaf Extract
(H and E stain, x 100 magnification). Showing normal capsular space (black arrow), regular glomerulus (red arrow)

4. DISCUSSION

The phytochemical composition of methanol extract of *M. charentia* showed that Flavonoids, Alkaloids Saponin, and Terpenoid were present. According to Kazmi *et al*., (2019) these secondary metabolites are known to be bioactive hepatoprotective agents. Saponins exhibit antimicrobial, immunostimulant, hypocholesterolaemic, anticarcinogenic activities and also defend plants against microbial pathogens (Desai *et al*., 2009). Alkaloids have several pharmacological activities including; antihypertensive, antiarrhythmic, antimalaria, anticancer and antiseptic effects (Debnath *et al*., 2018). Tannins serve as defense mechanism in plants against pathogens, herbivores and balance environmental conditions (Kaicome, 2005). Tannins generally causes a negative reaction when consumed, it can either cause decreased or increased growth rates , tannins retard rate of protein breakdown and removal of amide group resulting in lowering plasma urea nitrogen (PUN) and ammonia concentrations (Kaicome, 2005). Some

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phytochemicals are known phytotoxins that are toxic to animals; for example aristolochic acid causes cancer even at very low doses (Rietjens *et al*., 2005). Some phytochemicals are antinutrients that interfere with the absorption of nutrients. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts (Rietjens *et al*., 2005). As observed in the present study, the effect of *M. charentia* methanol leaf extract on albino rats might be attributed to these phytochemicals.

Serum alanine aminotransferase (ALT) is a cytoplasmic enzyme found in very high concentration in the liver and kidney, with skeletal muscles having lesser activity of the enzyme (Ozer *et al*., 2008), and serum increase of this enzyme indicates hepatocellular damage (Bala *et al*., 2012). Serum aspartate aminotransferase activity (AST) is located in the microsomal and mitochondrial portions of the liver cells, skin, skeletal and cardiac muscles, pancrease and kidney (Chigozie et al., 2016). Aspartate aminotransferase activity (AST) is less specific than ALT serves as detector of liver function and ALT activity is usually greater than AST activity at early or acute hepatocellular disease (Botros and Sikaris, 2013) although both are useful indices for identifying inflammation and necrosis of the liver (Goodman, 2007). Serum alkaline phosphatase (ALP) is increased in many clinical states; bone and liver diseases are the most important. Increase in ALP activity in liver disease is as a result of increased synthesis of the enzyme by cells lining the bile canaliculi, usually in response to cholestasis which maybe either intra or extra hepatic (Poupon, 2015). In this study, the AST, ALT and ALP activities of animals administered with oral doses of methanol extract of *M. charentia* were not altered indicating that there was no liver tissue damage as further supported by histological examination.

Total protein and albumin/globulin (A/G) ratio test measures the total amount of protein in blood and are made by the liver (Kumar *et al*., 2018). Decrease in albumin and total protein is a sign of reduced synthetic function of the liver. Low serum albumin content may suggest infection or continuous loss of albumin (Moshage *et al*., 1998). Bilirubin moves through the liver and is gradually excreted out of the body. Higher concentration of bilirubin above normal levels may indicate different types of liver or bile duct problems (Mohammed *et al*., 2012). As observed in the present study, there wasn't alteration in the serum concentration of both total and direct bilirubin in the groups administered with the extract when compared with the control indicating that the conjugating, excretory potentials of the liver was not affected.

Urea, uric acid, creatine, and creatinine are four major nonprotein nitrogenous compounds (NPN) components and are routinely determined in clinical settings (Yan *et al*., 1999). They are used to monitor renal function, High levels of uric acid are found secondary to a variety of diseases, e.g glycogen storage disease (Yan *et al*., 1999). Plasma levels of creatinine can reflect endogenous production and the glomerular filtration rate. Therefore, it is an excellent indicator for the assessment of renal function (Shea *et al*., 1981). In the present study there was no observable alteration in urea, uric acid and creatinine concentration suggesting that *M. charentia* methanol extract doesn't interfere with renal function

The total red blood cell count (RBC), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), haemoglobin (Hb) and packed cell volume (PCV) are the most useful indicators in the diagnosis of anaemia in humans and animals (Ukwuani-Kwaja *et al*., 2021). In this study, the evaluation of total RBC, MCHC, MCH, MCV, Hb and PCV did not altered following repeated administration of the extract when compared to control. These results showed that there is no lysis of blood cells, bleeding, anaemia and inhibition in blood cells synthesis White blood cell count increases in response to foreign substance as a defense mechanism of the body (Green *et al*., 1997). Increase in WBC count may be due to stimulation of WBC production increasing its availability in circulation in an attempt to defend the system (Rimmelé *et al*., 2016). According to Levin, (2019) platelets are fragments of cells and participate in blood clotting. None of the above mentioned parameters were altered after administering *M. charentia* methanol leave extract for 28days indicating that the plant doesn't heamatological parameters.

5. CONCLUSION

Momordica charantia, generally known as bitter melon, belongs to the family *Cucurbitaceae* and known over 100years for its pharmacological activities. In conclusion, with regard to the findings of the present studies the prolong oral administration of *M. charentia* methanol extract extract for 28days does not cause any toxic effect on liver enzymes, kidney function parameters, as well as heamatological parameter , hence the extract is relatively non-toxic and furthermore should be consider safe for consumption.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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